

# Cross-Canada Disease Report

## Rapport des maladies diagnostiquées au Canada

### Canada

#### First report of *Ureaplasma diversum*, a bovine pathogen, in the respiratory tract of swine in Canada

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*Ureaplasma* species have been largely considered as commensal or opportunistic pathogens inhabiting urogenital and respiratory tracts of vertebrate hosts. However, some *Ureaplasma* species such as *Ureaplasma urealyticum* and *Ureaplasma parvum* in humans (1) and *Ureaplasma diversum* in bovine species (2,3), have been recognized as important pathogens. Cattle diseases associated with *U. diversum* infection include seminal vesiculitis, balanoposthitis, and alterations in spermatozooids in bulls and pneumonia, placentitis, abortion and birth of weak calves, in cows (2–5).

*Ureaplasma diversum* in bovine species has been reported in several countries including Brazil (6,7), France (8), Canada (9,10), Costa Rica (11), Argentina (12), and Australia (13). In Canada, *U. diversum* has been identified in the reproductive tracts of cattle without clinical signs, in dairy cows with granular vulvitis (9,10) and in aborted bovine fetuses (14), showing lymphofollicular conjunctivitis and/or pneumonia and

in some cases, destructive polyarthropathy (5). Several cases of cow abortions from 2010 to 2016, in the province of Quebec (Canada), have been associated with *U. diversum*, with a significant increase in 2015 and 2016, as reported by the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) (15).

Identification of *U. diversum* can be done by bacterial isolation or by PCR assay. Bacterial culture is laborious and time-consuming while PCR assays are less time-consuming and more sensitive for detecting *U. diversum* in bovine clinical samples (16).

A new quantitative polymerase chain reaction (qPCR) diagnosis assay for the identification of *U. diversum* in bovine clinical samples was developed at the molecular diagnostic laboratory (MDL) of the Faculté de médecine vétérinaire (FMV) of the Université de Montréal, adapted from Marques et al (17). During the validation of this *U. diversum* qPCR diagnostic assay, 133 samples, including lung, nasal, and vaginal swabs, feces, milk, placenta, intestine, fetal tissues, synovial membrane and joint, serum and pools of tissues were used to determine the specificity of this assay. The samples came from various animal species, namely, pigs, cattle, cats, sheep, birds, dogs, goats, raccoons, and alpacas. Interestingly, 2 lung samples from pigs were found positive for *U. diversum* during the validation process. In Canada, this *Ureaplasma* species has never been reported in animal species other than cattle. To our knowledge, it has been reported once in swine worldwide and, more specifically, in Cuba (18). *Ureaplasma diversum* positive porcine lung samples came from 2 unrelated clinical cases which were submitted to MDL in 2015 and in 2016.

#### Case descriptions

Case 1 (FMV15-1774804). A boar from an insemination center was submitted for necropsy at the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) veterinary pathology laboratory in Quebec city, in June 2015 with a history of loss of appetite and a peak body temperature of 40°C. The diagnosis was a multicentric lymphosarcoma with gastric rupture and secondary peritonitis explaining the poor condition

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**Table 1.** Diagnostic assay results obtained from the 2 porcine *U. diversum* positive lung samples.

Pathogens	Case 1 <sup>a</sup>	Case 2 <sup>b</sup>	Type of diagnostic assay
PRRSV	—	+	qPCR
PCV2	nt	—	qPCR
Influenza A virus	—	—	qPCR
<i>Streptococcus</i> spp.	+	—	Bacterial culture
<i>Trueperella pyogenes</i>	+	—	Bacterial culture
<i>Actinobacillus indolicus</i>	+	—	Bacterial culture
<i>Haemophilus parasuis</i>	—	—	Bacterial culture
<i>Mycoplasma</i> spp.	+	+	PCR
<i>Mycoplasma hyopneumoniae</i>	—	+	qPCR
<i>Mycoplasma hyorhinis</i>	—	+	qPCR
<i>Ureaplasma diversum</i>	+	+	qPCR

<sup>a</sup> Case 1 — Boar from an insemination center subjected to necropsy on June 2015.

<sup>b</sup> Case 2 — Three to six-month-old fattening pig euthanized and submitted to necropsy on April 2016.

PCV2 — porcine circovirus type 2; nt — Not tested.

of this animal. However, there was also a broncho-interstitial pneumonia with bronchial-associated lymphoid tissue (BALT) hyperplasia, alveolar and interlobular edema, as well as infiltration of foamy macrophages arranged in small sheets, in some alveoli, strongly suggestive of a mycoplasma infection.

As requested by the pathologist, several diagnostic assays were performed but the results for these tests (Table 1) were not initially available to the FMV's employees who use a different computer system than do the MAPAQ's employees. Because qPCR for both *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* were negative, PCR for *Mycoplasma* spp. was requested, which interestingly, was found to be positive. Additional sequencing of the PCR amplicon was also requested and revealed 100% homology with *Ureaplasma* spp. which was subsequently confirmed to be *U. diversum* by a specific qPCR assay at Prairie Diagnostic Services (PDS) in Saskatoon.

Case 2 (FMV16-1864716). A 3- to 6-month-old fattening pig with a mycoplasma-type cough was euthanized and submitted to MAPAQ's veterinary pathology laboratory in Quebec city, in April 2016, less than a year after the submission of the previous case. The pig came from a herd of 1200 animals with a mortality of 2.08% and respiratory clinical signs affecting 33% of the pigs for 1 month. Similar pulmonary histological lesions, as described for case 1, were present. However, the lung sample was positive by qPCR for porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, *Mycoplasma hyorhinis*, as well as positive for *Mycoplasma* spp. Being aware at the MAPAQ laboratory of the previous *U. diversum* positive case, sequencing of *Mycoplasma* spp. PCR amplicon was performed, showing again the presence of *U. diversum*, but this time with 99% homology. Other tests were negative (Table 1).

## Results

During the validation of a new *U. diversum* qPCR assay at FMV, the lung samples from these 2 cases were randomly selected and were found to be positive. We were not aware of the previous results. For confirmation of our findings, the samples were submitted to a second diagnostic reference laboratory, PDS in Saskatoon, Canada, for detection of *Ureaplasma* spp. and *U. diversum* by PCR. They confirmed

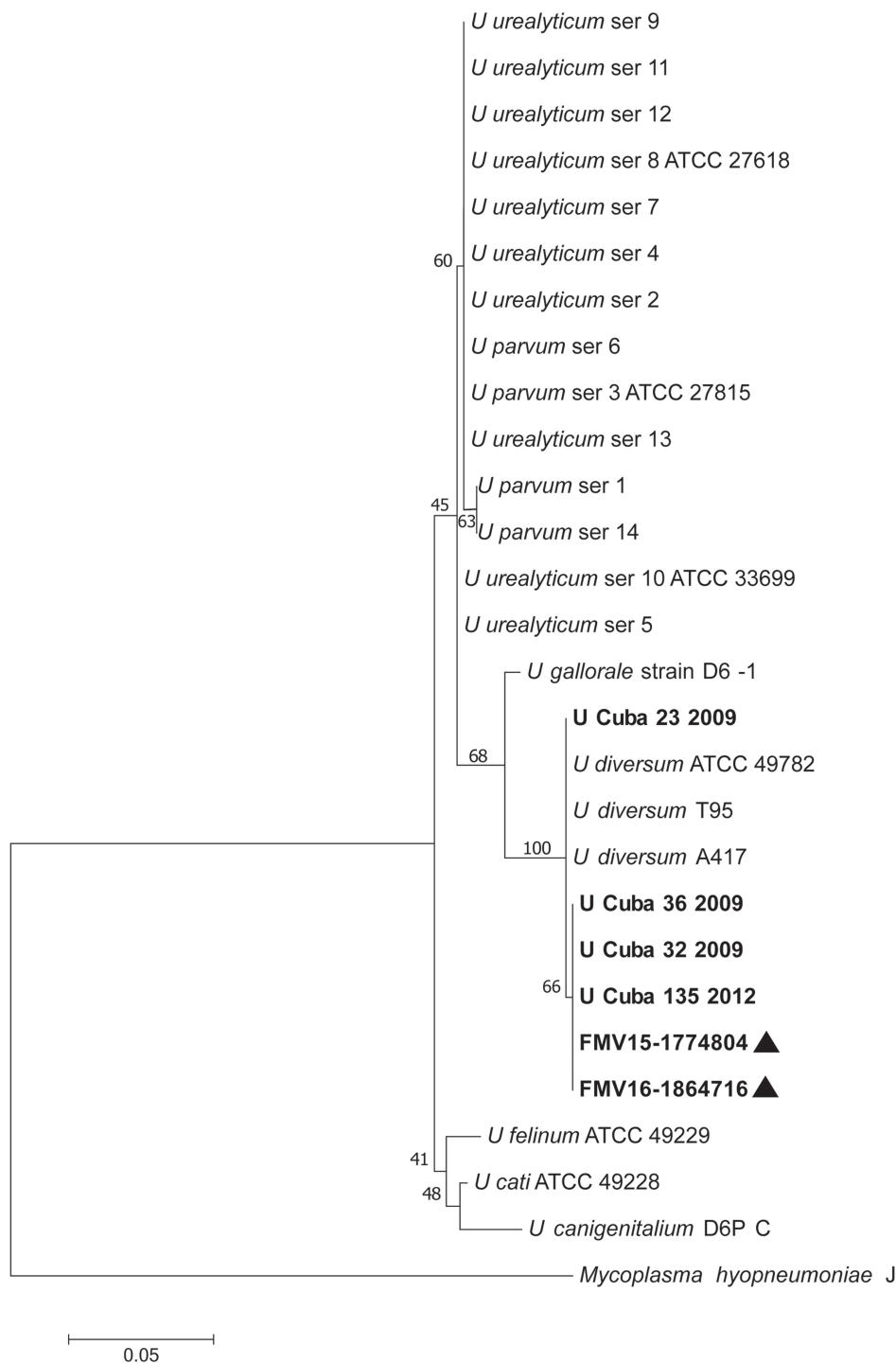
our positive results. Presence of *U. diversum* in these lung samples was also confirmed through sequencing of a PCR amplicon of 1037 nucleotides which was obtained using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and MGSO (5'-TGCACCATCTGTCACTCTGTTAACCT-3'). The PCR amplicon nucleotide sequences, which contain a partial sequence of the 16S rRNA gene, were deposited in the GenBank database (Case 1: strain FMV15-1774804; accession number MH428100) and (Case 2: strain FMV16-1864716; accession number MH428101). Nucleotide sequences obtained from both swine lungs showed 99% identity with *U. diversum* reference strain A417, as determined using the NCBI nucleotide Basic Local Alignment Search Tool (BLAST) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

*Ureaplasma diversum* was first reported in pneumonic lungs of swine in Cuba in 2014 (18). A phylogenetic analysis by Burgher et al (18) in 2014 demonstrated the genetic relatedness of the porcine Cuban ureaplasma strains with *U. diversum* species. A phylogenetic analysis was carried out to confirm the phylogenetic relatedness of our porcine ureaplasma strains with *U. diversum*, and particularly, to determine the genetic relatedness of the Canadian porcine ureaplasma strains with the Cuban porcine *Ureaplasma* strains. The partial sequences of the 16S rRNA of the Cuban and Canadian porcine *Ureaplasma* strains as well as of the following *Ureaplasma* species were included in this analysis: *U. urealyticum*, *U. parvum*, *U. cati*, *U. felinum*, *U. gallorale*, *U. canigenitalium*, and *U. diversum*. The nucleotide phylogenetic tree confirmed that the Canadian porcine ureaplasma strains were grouped within the *U. diversum* cluster. Interestingly, these porcine ureaplasma strains formed a sub-cluster with the Cuban porcine ureaplasma strains within the *U. diversum* cluster. The sequences of the porcine sub-cluster were 99% identical to the bovine *U. diversum* strains (Figure 1).

## Discussion

The pathogenic potential of *U. diversum* identified in swine remains unknown. However, a previous study conducted in Cuba reported a prevalence of *U. diversum* of 6.6% (7 of 106 porcine respiratory tract samples) (18). Moreover, all *U. diversum* positive samples reported in this previous study, were collected from pneumonic swine lungs. *Ureaplasma diversum* was not detected in any sample from the lungs of 13 healthy swine included in this study (18). Prevalence of *U. diversum* in the Canadian swine population is unknown. Therefore, 114 additional pig samples (76 lung, 36 pools of tissues, and 2 trachea) were selected randomly and subsequently tested by qPCR for *U. diversum*. Those samples, most of which originated from different swine facilities, were submitted to MDL to conduct several molecular diagnostic assays including porcine reproductive and respiratory syndrome virus (PRRSV) qPCR. Only 1 additional lung sample was found *U. diversum* positive, suggesting that the prevalence of *U. diversum* in lungs from pigs may be relatively low (i.e., 3/78 = 3.85%) (Table 2). However, a better experimental design is required to establish the prevalence of *U. diversum* in the Canadian swine population.

Respiratory disease in swine generally has a multifactorial origin. The combination of primary and opportunistic



**Figure 1.** Molecular phylogenetic analysis based on 16S rRNA partial nucleotide sequences of species of the genus *Ureaplasma*. The phylogenetic tree was constructed by the Maximum Likelihood method in the MEGA 7 package (24). The tree was scaled with branch lengths measured in the number of substitutions per site (bar, 0.05 substitutions per site). The bootstrap values presented at corresponding branches were evaluated from 1000 replications. The tree is rooted using the sequence of the strain *M. hyopneumoniae* J. FMV15-1774804 (GenBank accession number: MH428100) and FMV16-1864716 (GenBank accession number: MH428101) are the 16S rRNA partial nucleotide sequences from the porcine *U. diversum* strains identified in this study (▲). All sequences of porcine origin are highlighted in bold. Herein the GenBank accession numbers of the ureaplasma strains included into the nucleotide phylogenetic tree: NR 025878 (*U. diversum* A417); CP009770 (*U. diversum* ATCC 49782); JN935894 (*U. diversum* T95); U06096 (*U. urealyticum* ser 2); AF073447 (*U. urealyticum* ser 4); AF073448 (*U. urealyticum* ser 5); AF073449 (*U. urealyticum* ser 7); AF073450 (*U. urealyticum* ser 8 ATCC 27618); AF073451 (*U. urealyticum* ser 9); CP001184 (*U. urealyticum* ser 10 ATCC 33699); AF073453 (*U. urealyticum* ser 11); AF073454 (*U. urealyticum* ser 12); AF073455 (*U. urealyticum* ser 13); AF073458 (*U. parvum* ser 1); CP000942 (*U. parvum* ser 3 ATCC 27815); AF073459 (*U. parvum* ser 6); AF073457 (*U. parvum* ser 14); NR 026027 (*U. gallorale* strain D6 -1); HM135464 (*U. felinum* ATCC 49229); HM241738 (*U. cati* ATCC 49228); NR 025877 (*U. canigenitalium* D6P C); KC686352 (U Cuba 36 2009); KC686353 (U Cuba 32 2009); KC686354 (U Cuba 23 2009); KC686355 (U Cuba 135 2012) and AE017243 (*Mycoplasma hyopneumoniae* J).

**Table 2.** Prevalence of *U. diversum* qPCR positive porcine samples.

Type of sample	<i>U. diversum</i>		
	Number of samples tested	Number of samples positive	% of samples positive
Lung	78	3 <sup>a</sup>	3.85%
Trachea	2	0	0%
Pool of tissues <sup>b</sup>	36	0	0%
Total	116	3	2.59%

<sup>a</sup> Includes the 2 *U. diversum* positive cases described in the manuscript (i.e., FMV15-1774804 and FMV16-1864716).

<sup>b</sup> No description of the type of tissues within the pools was provided by the veterinarians who submitted the samples to MDL.

infectious agents is a common finding during respiratory disease outbreaks in pig farms (19). The *U. diversum* positive porcine lung samples were also positive for *Mycoplasma* spp. (Table 1). Based on bioinformatic analyses, our *Mycoplasma* spp. PCR diagnostic assay may detect several species of mycoplasmas and ureaplasmas including *U. diversum*. Therefore, both *U. diversum* positive lung samples may also be co-infected by other mycoplasma and ureaplasma species. In fact, the lungs of the second case were positive for *M. hyopneumoniae*, *M. hyorhinis* in addition to PRRSV (Table 1). *Mycoplasma hyopneumoniae* and PRRSV are common primary infectious agents found in the respiratory tracts of swine. This is particularly the case with *M. hyopneumoniae*, which is the primary pathogen associated with enzootic pneumonia. It colonizes the respiratory tract of pigs by adhering to the cilia, leading to reduced function of the mucociliary apparatus (20). This allows the respiratory tract of pigs to be colonized by secondary bacterial pathogens. Porcine reproductive and respiratory syndrome virus is one of the most economically important pathogens of swine, responsible for both reproductive and respiratory diseases. It is the most common virus isolated from cases of Porcine Respiratory Disease Complex (PRDC) (21). Some studies demonstrated that *M. hyopneumoniae* enhances the severity and duration of PRRSV-induced pneumonia (22). There is also evidence of the association of PRRSV with outbreaks caused by other pathogens (21). It is believed that in addition to the pathogens known to induce respiratory disease, several emerging pathogens could play a role in the development of PRDC (21). Therefore, further studies will be necessary to determine whether *U. diversum* could be an opportunistic pathogen or a primary agent involved in PRDC.

To our knowledge, this is the first report of *U. diversum* in the respiratory tract of swine in Canada. However, the present study has the limitation that the classification and phylogeny of the porcine *U. diversum* was only based on the 16S rRNA nucleotide sequence. There are several examples in which different species of the family *Mycoplasmataceae*, including *Ureaplasma* species, share 99% sequence identities based on 16S rRNA sequence analyses (23). This latest data support the need to confirm the phylogenetic results by others means. In that regard, high throughput sequencing analyses are being conducted to obtain the complete genome sequence of the porcine *U. diversum* strains.

*Ureaplasma diversum* is a well-recognized bovine pathogen that can cause various clinical signs in infected cattle. For this

reason, we believe that it is important to inform the Canadian swine industry stakeholders of its existence in the respiratory tract of swine.

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## Answers to Quiz Corner Les réponses du test éclair

1. C) Wild cats are susceptible to FIP.  
C) Les chats à l'état sauvage sont sensibles à la PIF.
2. A) Titers to *E. canis* do not protect against reinfection.  
A) Les titres de *E. canis* ne protègent pas contre la réinfection.
3. A) Struvite uroliths form in alkaline urine. Acidic urine is helpful in the prevention of struvite uroliths.  
A) Les urolithes de struvite se forment dans l'urine alcaline. L'urine acide est utile pour prévenir les urolithes de struvite.
4. A) Cloacal prolapse is often multifactorial. A cloacopexy surgery to pull the cloaca back into position is commonly performed; if the factors that led to the prolapse are not corrected, the cloacopexy is likely to fail once the sutures from the surgery are resorbed.  
A) Le prolapsus du cloaque est souvent multifactoriel. Une cloacopexie pour remettre le cloaque en position est communément effectuée. Si les facteurs qui causent le prolapsus ne sont pas corrigés, il y aura possiblement échec de la cloacopexie lorsque les sutures chirurgicales seront résorbées.
5. B) Laminitis in cows is usually mild and dissimilar to the equine presentation. Lameness can ensue, but it is due to secondary complications from changes in hoof integrity (e.g., white line separation, sole abscesses, sole ulceration). One of the first visible signs is hemorrhage within the sole horn, when that horn grows close to the surface. Also, serum can discolor the horn yellow and soften it. Fever is not a symptom of bovine laminitis. Pedal rotation does occur, but never results in the phalanx sinking through the sole.  
B) La fourbure chez la vache est habituellement légère et ne ressemble pas à celle chez le cheval. Elle peut produire une boiterie, mais elle est due à des complications secondaires à la suite de changements dans l'intégrité de l'onglon (p. ex., séparation de la ligne blanche, abcès de la sole, ulcère de la sole). L'un des premiers signes visibles est une hémorragie dans la corne de la sole, lorsque cette corne pousse près de la surface. Aussi, le sérum peut décolorer la sole et l'amollir. La fièvre n'est pas un symptôme de la fourbure chez la vache. La rotation de la phalange distale peut se produire, mais ne produit jamais une perforation de la sole par la phalange.